FF 'A' letter

American Red Cross

Biomedical Research and Development
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Harmon C. McAllister, Ph.D.
Research Director
The Council for Tobacco Research-U.S.A., Inc.
900 Third Avenue
New York, NY 10022

Dear Dr. McAllister:

Enclosed please find a brief outline of a research proposal which I would like to present to the Tobacco Research Council for consideration. A detailed application for funding will follow if requested by the Council.

The platelet, in addition to its obvious importance in thrombosis, is an extremely useful model for studies of intracellular signal transduction. Recent work has identified platelet receptors for collagen and thrombin. The receptor for ADP has yet to be identified. The specific pathways by which receptors for the various agonists mediate the events which lead to platelet aggregation are not completely understood. Almost nothing is known about the signaling events which immediately follow receptor occupancy.

Recent studies in my laboratory have characterized the compound phenylarsine oxide (PAO) as a potent inhibitor of the activation and aggregation of platelets. PAO is effective against a broad spectrum of agonists including thrombin, collagen, ADP, epinephrine, arachidonic acid, calcium ionophores and phorbol myristate acid and is effective at concentrations as low as 100nM. Inhibition of all of these agonists of platelet aggregation suggests the PAO acts by interfering with one or more mechanisms basic to the activation and subsequent aggregation of platelets. I have recently discovered that PAO induces the phosphorylation of a specific subset of platelet proteins. This observation may be explained by PAO inhibition of a specific phosphoprotein phosphatase.

PAO is know to react with proteins containing adjacent sulfhydryl groups. A most interesting aspect of PAO inhibition of platelet aggregation is that the inhibition can be reversed by dimercaptopropanol (DMP). DMP, which was developed during World War II as an antidote to arsenic-based poison gas, contains two closely spaced sulfhydryl groups and effectively competes for and removes protein-bound PAO. I have utilized DMP to rapidly and completely recover platelet responsiveness 24 hrs after inhibition of PAO.

The detailed proposal which I would like to present to the Council would contain three specific aims:

1) To identify platelet proteins which are modified by PAO.

2) To identify and characterize the proteins which are phosphorylated in the presence of PAO and the protein kinases and/or phosphoprotein phosphatases involved.

3) To identify and/or synthesize PAO analogs which can be used therapeutically.

The final research proposal would be for three years of support with an annual budget of approximately $90,000.00/year for support of one technical position, one postdoctoral scientist and 25% of the Principal Investigator's salary.

The importance of elucidation of the mechanism(s) of action of PAO are twofold: 1) It is hypothesized that PAO will serve as an important tool with which to probe basic and fundamental mechanisms of platelet signal transduction. 2) Identification of the mechanism of action of PAO could lead to the design of a therapeutically useful analog of PAO that could be used in the treatment of the thrombotic complications found in a variety of diseases and surgical procedures.

The possible ramifications of this work are very exciting. Please contact me at your convenience if you have questions regarding the appropriateness of this proposal for Council consideration.

Sincerely,

Dale E. Greenwalt

Dale E. Greenwalt, Ph.D.
Scientist I
Cell Biology

DEG/lm